

## The impact of drying and rewetting of leaf litter on feeding activity of *Bibio pomonae* (Diptera, Bibionidae) larvae

[Der Einfluß von Trocknung und erneuter Anfeuchtung von Pappelblattstreu auf die Fraßaktivität der Larven von *Bibio pomonae* (Diptera, Bibionidae)]

by  
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**Abstract** Food consumption, defecation and production rates were investigated in *Bibio pomonae* larvae reared on poplar (*Populus nigra*) leaf litter. Drying and rewetting of leaf litter affected all investigated parameters of feeding activity negatively, in comparison with leaves kept in original moisture. Leaching, changes in microbial assemblages and lower moisture of dried and rewetted litter are probable reasons for these differences.

**Key words** consumption, defecation, production, soil fauna

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**Zusammenfassung** Die Konsumptions-, Defäkations- und Produktionsraten von *Bibio pomonae* - Larven wurden in Laborexperimenten bestimmt. Als Nahrungssubstrat diente Pappelblattstreu (*Populus nigra*) im natürlichen Zustand und solche, die zunächst getrocknet und dann wieder angefeuchtet worden war. Larven, die mit unbehandelter Streu gefüttert wurden, fraßen mehr, gaben mehr Kot ab und wuchsen schneller als die, denen behandelte Streu angeboten wurde. Die verminderte Eignung der behandelten Streu als Nahrungssubstrat ist vermutlich auf das Auswaschen von Nährstoffen, eine Reduktion des Besatzes mit Mikroorganismen und darauf zurückzuführen, daß durch die Wiederbefeuchtung der natürliche Feuchtigkeitsgehalt der Blätter nicht wiederhergestellt werden kann.

**Stichwörter** Konsumtion, Defekation, Produktion, Bodenfauna

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### Introduction

In certain terrestrial ecosystems bibionid larvae are important decomposers of plant litter (D'ARCI BURT & BLACKSHAW 1991, KARPACHEVSKIJ et al. 1968). They are able to consume a significant part of the annual litter fall (SZABÓ 1974). The study of their feeding activity is important to evaluate the total matter turnover in ecosystems. The extrapolation of laboratory data to field conditions brings some difficulties. The ingestion of own fecal pellets can distort the value of consumption estimated in laboratory conditions (DANGERFIELD 1994). Other difficulties derive from the changes of food quality in laboratory experiments. Dried and rewetted food is commonly used in the studies of feeding activity of litter dwelling invertebrates (e.g. GERE 1962, FADIEL & NAIR 1991). Drying and rewetting affected significantly some chemical parameters of plant litter: nutrients and phenolic compounds are lost due to leaching (GUNNARSSON et al. 1988, IBRAHIMA et al. 1995). The activity of microorganisms changed after drying and rewetting (CLEIN & SCHIMEL 1994). However the impact of drying and rewetting on the feeding activity of litter dwelling invertebrates has not been studied yet. The main question of this study is: what is the impact of drying and rewetting of the plant litter on the feeding activity of *Bibio pomonae* larvae?

## Material and methods

**Material:** *Bibio pomonae* FABRICIUS, 1775 larvae were taken from poplar (*Populus nigra*) forest near České Budějovice (380 m a.s.l., mean annual temperature 7.8 °C, annual precipitation 620 mm) on 15 September 1993 (first experiment), and on 7 November 1994 (second experiment). Fresh mass and dry mass content of larvae used in the experiments are summarized in Tab. 1. The fresh mass of larvae used was considerably lower than the maximal fresh mass of *Bibio pomonae* larvae shortly before pupation (0.1200 g), thus we assumed that the larvae were rapidly growing in both experiments. Partially fermented (black) fallen leaves from the same place where the larvae were collected were used as food. The fresh leaves and animals were stored for one week before the experiment under the experimental conditions. Animals fed on fresh leaves ad libitum. The leaves used in the experiments were superficially cleaned from large organic and mineral particles and the middle ridges were removed. The remaining leaf areas were cut to pieces (1 x 2 cm). Dried and rewetted leaves and leaves kept in original moisture were used. The former were prepared by rewetting air-dried leaves by immersion in water. The redundant water was soaked by placing on a filter paper. The samples of air-dried leaves were oven-dried to determine their dry matter content. All organic materials were oven-dried up at 60 °C for 24 hours. The dry mass of leaves kept in original moisture was calculated from their fresh mass and the dry matter content of a separate sample.

**Experimental design.** In the first experiment four types of food were compared: leaves in original moisture and air-dried leaves rewetted for 10 and 60 minutes and 24 hours. Six larvae and fresh or rewetted leaves (c. 1g of dry mass) were placed in plastic Petri dishes (5 cm six repetitions per variant). The amount of food used ensure that substantial majority of food (c. 80%) remain unconsumed after 7 days and thus consumption was not food limited. The Petri dishes were placed into a box with a plaster bottom soaked with water. The box was situated in a thermostat (15 °C, 95-100% RH, LD 0/24). After 7 days, fresh and dry masses of larvae were measured, and the remainders of leaves and excrements were separated, dried and weighed. Whereas in first experiment four groups of larvae were studied parallelly in second experiment one group of larvae were used and two treatment of food were compared in two following time period. In the second experiment leaves in original moisture and dried leaves rewetted for 24 hours were compared. The experiment consisted of six 7 - day intervals. Rewetted leaves were used as food during the first three intervals, leaves in the original moisture were used in the second three intervals. At the beginning of each interval the known amount of leaves was added and at the end of each interval the dry masses of the remaining leaves and excrements were measured. The fresh mass of larvae was determined in each repetition at the beginning and at the end of each interval. Dry mass contents of larvae were established three times: at the start of the first interval (from a separate sample), after the third interval and at the end of the experiment. In all cases 20 larvae were used. Eight repetitions each with eight larvae were used during the first three intervals. Eight repetitions each with five larvae were established for the second half of the experiment. Experimental conditions were the same as in the first experiment. In each interval five Petri dishes with leaves only were used as controls.

**Data processing.** Consumption (C) was calculated as the difference between the dry mass of the leaves at the beginning (LB) and at the end (LE) of an interval. The dry mass at the beginning of an interval was reduced by a correction factor (c) accounting for dry mass losses in controls (caused by microbial breakdown, leaching, crushing of fine particles, etc.). This fac-

tor was calculated as the dry mass of the control at the end of an interval, divided by the dry mass of the control leaves at the beginning of the interval. Thus consumption was calculated as  $C = LB \cdot c - LE$  and related to mean dry mass of larvae and day [ $g \cdot g^{-1} day^{-1}$ ]. In the first experiment the correction for mass loss in control was not used. Defecation rate was expressed as the dry mass of excrements produced per mean dry mass of larvae and day [ $g \cdot g^{-1} day^{-1}$ ]. Assimilation (A) was calculated as the difference between consumption and defecation [ $g \cdot g^{-1} day^{-1}$ ]. Production rate (P) was calculated as the difference of larval dry mass at the beginning (AB) and at the end (AE) of an interval divided by the dry mass of larvae at the beginning of the interval and the length of the interval [ $g \cdot g^{-1} day^{-1}$ ]. Assimilation and production efficiencies were calculated as  $A/C$  and  $P/C$ , respectively. The differences between two groups were tested by t-test, the differences among 3 groups were tested by ANOVA, STUDENT NEWMAN-KEULS test (SKN) was used for comparison of means. Average values and standard deviation are expressed in form average  $\pm$  SD, r means linear correlation coefficient.

## Results

### First experiment

The consumption, defecation and production rates were higher in the variant with original leaves than in all rewetted leaves (Fig. 1). These differences were most apparent in consumption rates. The consumption rate was 1.94 times higher for larvae fed original leaves than for larvae fed rewetted leaves ( $P = 0.05$ , t-test). The production rate of larvae fed original leaves was significantly ( $P = 0.05$ ) higher than larvae fed on rewetted leaves. Differences in defecation was not significant. Food consumption rates were negatively correlated with food dry matter content ( $r = -0.587$ ,  $P = 0.01$ , calculated from both the rewetted and original variants; see Tab. 1 and Fig. 1). The consumption was significantly ( $P = 0.05$  one way ANOVA, SKN test) lower on leaves rewetted for 10 minutes compared to leaves rewetted for longer duration. The production of larvae fed rewetted leaves increased in a row 10 minutes < 24 hours < 60 minutes, the differences in all these variants were significant ( $P = 0.05$ , one way ANOVA, SKN test). The assimilation efficiency varied from 59.8 % in the leaves rewetted for 10 minutes to 76.0% in the original leaves (Tab. 2).

The production efficiency reached 13.8 % in the original leaves, but in all treatments using rewetted leaves the larvae lost weight (Tab. 2).

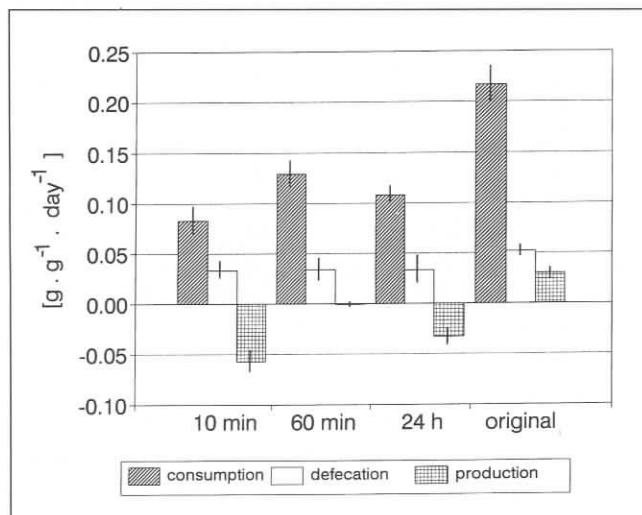


Fig. 1: Food consumption, defecation and production rates of *Bibio pomonae* FABR., 1775 larvae on dried poplar (*Populus nigra*) leaf litter rewetted for 10 and 60 minutes and 24 hours and litter kept in original moisture. Bars represent standard deviation,  $n = 6$ .

**Tab. 1:** Body mass of *Bibio pomonae* FABR., 1775 larvae and dry mass content of larvae and leaves (*Populus nigra*) used in experiments

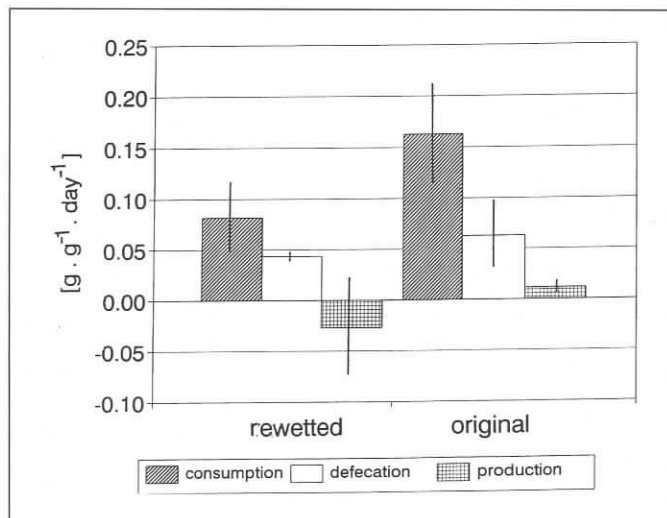
		fresh body mass larvae [mg]	dry matter content larvae [%]    leaves [%]	
first experiment	rewetted 10 min	27.2±3.1	19.60±7.10	46.50±5.98
	rewetted 60 min	19.2±2.5	19.60±7.10	39.23±5.04
	rewetted 24 h	23.4±4.0	19.60±7.10	40.20±6.64
	original	27.2±5.3	19.60±7.10	38.15±3.72
second experiment	rewetted	84.4±8.1	27.92±1.60	30.15±3.37
	original	87.3±13.9	25.49±5.80	22.43±2.02

**Tab. 2:** Assimilation rates and assimilation and production efficiencies of *Bibio pomonae* FABR., 1775 larvae. A = assimilation [ $\text{g} \cdot \text{g}^{-1} \cdot \text{day}^{-1}$ ], A/C = assimilation efficiency [%], P/C = production efficiency [%], - production efficiency for negative value of production was not calculated

		A	A/C	P/C
first experiment	rewetted 10 min	0.0496	59.8	-
	rewetted 60 min	0.0956	73.8	-
	rewetted 24 h	0.0745	68.9	-
	original	0.0648	76.0	13.8
second experiment	rewetted	0.0367	46.0	-
	original	0.0997	61.4	7.0

### Second experiment

All parameters measured were higher in the original than in the rewetted leaves (Fig. 2). The consumption was 1.94 times higher in the original than in the rewetted leaves ( $P = 0.001$ , t-test). The production rate was positive in the larvae fed original leaves, loss of weight appear in larvae fed on rewetted leaves and the differences were significant ( $P = 0.025$ , t-test), the smallest difference was observed in the defecation rates ( $P = 0.05$ , t-test). Assimilation and production efficiencies show similar pattern as in first experiment but the values were lower (Tab. 2). Influence of rewetting on weight loss in control: The mass loss of original leaves

**Fig. 2:** Food consumption, defecation and production of *Bibio pomonae* Fabr., 1775 larvae. First three intervals when the larvae fed on dried and rewetted poplar (*Populus nigra*) leaf litter, are noted as rewetted and next three interval, when the larvae fed litter kept in original moisture, are noted as original. Bars represent standard deviation,  $n = 24$ .

found in the controls of the second experiment ( $1.06 \pm 4.30\%$ ,  $n = 15$ ) was significantly ( $P = 0.001$ , t-test) lower than in the rewetted leaves ( $14.56 \pm 5.96\%$ ,  $n = 15$ ). Despite the differences in the mass loss of both controls (original and rewetted), the variability of the mass loss of controls in both treatments was not significantly different (F-test).

### Discussion

The values of consumption, defecation and production rates were similar to the values given by DELEPORTE (1988) for *Bradysia confinis* WINNERTZ, 1867 larvae, and consumption rates for *Bibio pomonae* agree well with those of *Bibio marci* LINNÉ, 1758 (POBOZSNY 1982). The assimilation and production efficiencies found in our experiment were higher than in other litter feeding invertebrates (GERE 1962, FADIEL & NAIR 1991, SCHAEFER 1990). However the assimilation and production efficiencies well agree with the data given for *Bradysia confinis* larvae (DELEPORTE 1988). The production efficiency observed agree with those given by SZABÓ (1974) for *Bibio marci*. All parameters of the feeding activity studied were reduced in the dried and rewetted treatments compared to original leaves in both experiments. The reduction was particularly drastic in the case of production rates which were negative in all rewetted treatments. A positive correlation between food consumption rate and the litter moisture was found in the first experiment. The moisture of rewetted leaves was low compared to that of original leaves (Tab.1) in both experiments. This difference in the moisture can be the reason for the observed decrease of the feeding activity. But moisture is probably not the only factor affecting the feeding activity after drying and rewetting. Intensity of feeding activity was similar in both experiments, despite the rewetted leaves in the second experiment contain more water than the original leaves in the first experiment (Tab.1). Thus the changes of litter quality due to drying and rewetting, particularly leaching and the changes in litter microbial community can play a role in the observed reduction of feeding activity, too. A significant amount of litter dry mass can be loosed by leaching (e.g. loss of 24% of initial dry mass during the first day is reported for *Populus tremuloides* (PARSON et al. 1990). In initial phase of leaching mostly easy utilizable substances (soluble sugar etc.) are leached (IBRAHIMA et al. 1995) and relative content of cellulose and lignin increase. This can decrease palatability of leaf litter, but leaching can have negative as well as positive effects on the feeding activity because not only nutrients but also some plant chemicals inhibiting feeding are leached (GUNNARSSON et al. 1988). The changes of microbial assemblages may be another important factor. In agreement with CLEIN & SCHIMEL (1994) a significantly higher microbial breakdown was observed in the rewetted treatments. Moreover, changes in the composition of the microbial community are expected. It is necessary to consider that the changes in litter microbial assemblages caused by rewetting in the laboratory are different from those occurring in nature (CLEIN & SCHIMEL 1994). A substantial part of microbial assemblages is killed by drying. In nature, rewetted leaves can be quickly recolonized from the surrounding soil or litter layer, but this is not possible in the laboratory. This can result in the changes of microbial assemblages. The rapid decrease of food consumption rates in larvae fed dried and rewetted leaves indicated a reduced palatability of leaves. This might be caused by the loss of nutrients due to leaching and/or by the changes of microbial assemblages. Reduced palatability may also be caused by low water content of rewetted leaves. The variability of experimental data depends not only on the variability of feeding activity itself but also on the background variability caused by the variability in the microbial breakdown, the variability in the moisture of the litter, the variability in the mass loss by leeching during rewetting, etc. The variability of control dishes (without larvae) was used to compare background variability between the rewetted and the original leaves. Although the dry mass

loss in control dishes differed substantially between rewetted leaves and leaves kept in original moisture, the variability of these parameters was not significantly different. We can conclude that the background variability and thus the accuracy of both experimental designs, is approximately the same. Nevertheless, the main sources of the background variability are probably different. In the dried and rewetted leaves the increase of microbial breakdown and leaching seems to be the most important. On the other hand a litter moisture variability which affects the calculation of the initial dry mass of leaves plays probably the most important role in leaves kept in natural moisture. Generally the using of leaves kept in original moisture can be recommended in feeding experiments. This design does not reduce the accuracy of measurement and results obtained seems to be more representative for natural condition than in case of dried and rewetted leaves. Differences observed between the both experimental designs indicated that width using of design with original leaves can bring a significant correction of knowledge about role of saprophagous invertebrates in organic matter turnover.

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